Appendix A: Supporting Information

Host macrophage response to injectable hydrogels derived from ECM and α-helical peptides

Authors

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Additional Figures

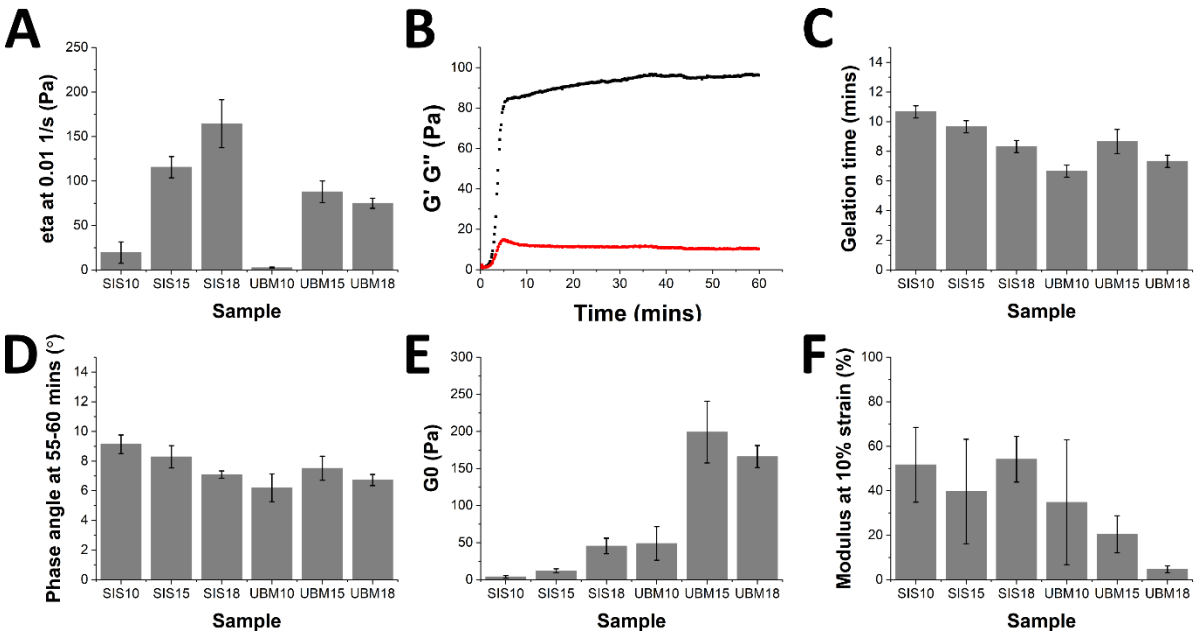

Figure S1 Rheological properties of SIS and UBM at 10 mg/mL, 15 mg/mL and 18 mg/mL show (A) the viscosity of solutions before gelation, (B) a representation of gelation kinetics depicted by G' (black) and G" (red), (C) the time taken for gelation to occur across all samples, (D) the ratio of viscous to elastic behaviour in the hydrogel, (E) the viscoelastic properties of the hydrogels determined through a Generalised Maxwell Model and (F) the modulus of the hydrogels at 10% strain.

Materials and Methods

Rheometry

Rheological tests were performed as previously described. Briefly, an AR2000 rheometer (TA instruments, New Castle, DE) fitted with a 40 mm parallel plate geometry and a Peltier plate was used to perform all tests. Data was analysed using the American Society for Testing and Materials (ASTM) standard F2900-11 (Guide for characterisation of hydrogels used in regenerative medicine). The Peltier plate was cooled to 10 °C and the hydrogel solution was loaded onto the rheometer. After

setting a gap of 750 µm between the upper and lower platen, mineral oil was applied to seal the edges of the sample-plate interface to minimise evaporative loss.

All tests were conducted in sequence for each sample and in triplicate. For each concentration of the SIS and UBM solutions, the steady state viscosity, η, as a function of shear rate, $\dot{\gamma}$, was measured. The data was fitted to a power-law model, Eq. 1, providing values for the consistency, k, and power-law index, n; these parameters were used to calculate the viscosity at a shear rate of 0.01 s^{-1}.

A low-amplitude oscillatory time sweep was performed to measure the gelation kinetics of the forming ECM hydrogel by rapidly raising the temperature to 37 °C (a temperature at which ECM gelation occurs) and applying a 0.5% oscillatory strain, γ, at a frequency, ω, of 1 rad s^{-1}. The storage modulus, G', and loss modulus, G'', were recorded for 60 minutes. The phase shift, δ, was calculated from the acquired data according to Eq. 2. The gelation time, t_{gel}, was considered to be the time after which the phase shift varied by no more than ±0.2°.

After gelation a low-amplitude oscillatory frequency sweep was performed to measure the complex viscosity, $|\eta^*|$, of the gelled material. The frequency range employed was 0.1–100.0 rad s^{-1} under the application of 0.5% oscillatory strain. This data was fitted to a three-element generalized Maxwell model, Eqs. 3-4, and the modulus of the gelled material, G_0, was determined using Eq. 5. The viscosity and relaxation time for each element are η_i and λ_i respectively.

The strain-dependent viscoelastic response of the gelled material was assessed at an oscillation frequency of 1 Hz. The data was fitted to a damping function, $h(\gamma)$, of the form shown in Eq. 6, in which α and β are parameters fitted to the measured strain-dependent behaviour of the liquid. For the purpose of meaningful comparison between samples, the percentage modulus at 10% strain was calculated. This analysis assumes that the effects of strain and strain rate are independent.

$$\eta(\dot{\gamma}) = k\dot{\gamma}^{n-1} \quad \text{(Eq. 1)}$$

$$\delta = \tan^{-1}\frac{G''}{G'} \quad \text{(Eq. 2)}$$

$$G'(\omega) = \sum_{i=1}^{N} \frac{\eta_i \lambda_i^2 \omega^2}{1+(\lambda_i \omega)^2} \quad \text{(Eq. 3)}$$

$$G''(\omega) = \sum_{i=1}^{N} \frac{\eta_i \lambda_i \omega}{1+(\lambda_i \omega)^2} \quad \text{(Eq. 4)}$$

$$G_0 = \sum_{i=1}^{3} \frac{\eta_i}{\lambda_i} \quad \text{(Eq. 5)}$$

$$h(\gamma) = \frac{1}{1+\alpha\gamma^{\beta}} \quad \text{(Eq. 6)}$$

Results

While all materials examined in this study formed hydrogels, rheological studies were performed to further characterise the mechanical and material properties of the hydrogels. Specifically, the concentration of SIS and UBM hydrogels was modified to match the stiffness of 1 mM hSAF hydrogels (1 kPa). These data showed that while viscosity increases as the SIS concentration is increased, as expected with the presence of increased fibre entanglements, UBM revealed complex rheological behaviour that did not fit this trend (**Figure S1A**). Despite the microstructural differences between the two hydrogel types both SIS and UBM displayed viscoelastic behaviour consistent with gelation (**Figure S1B**). Both hydrogel solutions reached a stable G' value, indicative of complete gelation, by 12 mins (**Figure S1C**) with the ratio of viscous to elastic forces approximately 8-10°, further evidencing a more 'solid-like' gel structure (**Figure S1D**). The trends observed for SIS and UBM, as a function of concentration, are also reflected in the modulus of the two materials (**Figure S1E**).

Further differences between the two ECM hydrogels; SIS and UBM, were noted in their tensile properties with 10 mg/mL SIS losing more than 50% of its strength at 10% strain. By comparison a rapid decrease in strength with increasing strain was observed for UBM (**Figure S1F**). Collectively, these studies show a difference in the complex rheological behaviour of SIS and UBM, which although not examined further here, did reveal that 8 mg/mL SIS and 15 mg/mL UBM hydrogels had a stiffness equal to that of 1 mM hSAF hydrogels and 8 mg/mL collagen hydrogels. Therefore, these concentrations were used for all other studies presented.

References

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